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THE PERIPHERAL INTERACTION OF CORTISONE AND THYROXINE AS REFLECTED BY NITROGEN BALANCE IN THE STRESSED, THYRO-PARATHYROIDECTOMIZED, ADRENALECTOMIZED RAT

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by

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A Thesis submitted to the Faculty of Yale University in partial fulfillment of the requirements for the degree Doctor of Medicine.

Department of Surgery

1955

ACKNOWLEDGMENT

I wish to thank Dr. M. A. Hayes for his generous advice, for providing laboratory facilities in the Samuel Harvey Metabolic Laboratory, and for adequate funds to support this project. Without Dr. Hayes' advice and support this experiment would have never reached completion.

I also wish to thank Dr. R. Cooke for allowing the housing of the experimental animals in his animal room.

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The Peripheral Interaction of Cortisone and Thyroxine as Reflected by Nitrogen Balance in the Stressed, Thyro-parathyroidectomized, Adrenalectomized Rat

That the production of the hormone of both the thyroid gland and the adrenal cortex is modulated by a trophic hormone of the anterior pituitary gland is not doubted. It is the interaction of the end product of these glands with one another peripherally and on the secreting glands themselves that remains somewhat enigmatic.

The thyroid hormone, probably thyroxine (1), in the absence of the cortical hormones increases the basal metabolic rate (2) and increases protein catabolism. (3) (6) Administration of the thyroid hormone to a normal animal causes the adrenal glands to enlarge in both size and weight. This hypertrophy is interpreted to mean an increase in cortical function. (3-5) Exposure to cold increases the utilization of the thyroid hormone and also its secretion. In a similar fashion, increased temperature lessens the demand for thyroid hormone. (7-10)

The steriods of the adrenal cortex influence the intermediary metabolism of many compounds: salt and water by the kidney (11) (18-20), carbohydrate (11)(15)(48), protein (11-14)(17-19), and fat (11)(21-22).

Cortical steroid insufficiency is associated with hypoglycemia. "Carbohydrate utilization thus appears to be proceeding at a rate which is excessive in terms of the availability of carbohydrates from preformed stores and by gluconeogenesis. The major defect in gluconeogenesis appears to be related to the difficulty in mobilizing endogenous protein for catabolism".(11) Experimental data would indicate that the defect in protein catabolism is at the level of the whole protein, involves not only a decreased protein catabolism but also an increased protein anabolism, and is extra hepatic. (11-14)

When present in excess, the cortical steriods produce a negative nitrogen balance and may be associated with glycosuria. (15-19) The negative nitrogen balance of cortical hormone excess can be diminished or completely reversed by the administration of glucose. (12)(14)(41)(44) It has been reported that the cortical hormone liberates nitrogen primarily from the lymphoid tissue. (42) That cortical hormone releases carcass nitrogen as well as that of the lymphoid tissue has been reported. (14) That the cortical hormone acts on such specific protein sources rather than in a non-specific manner on all protein has been questioned. (17)

Studies with I_{131} have shown that the thyroid uptake of radioiodine is depressed following treatment with adrenal cortical extract or adrenocorticotrophic hormone. (23-30) Other investigators have not found a decreased uptake of radioiodine after cortisone therapy. (31-34) Histologic studies have shown that cortisone does not inhibit the formation of thyroid stimulating hormone, does not inhibit the response of the thyroid gland to TSH.

(33)(35)(45-46) Release of thyroid hormone is not blocked by cortisone. (36-38) (23)(47) An increased renal excretion of radioiodine following cortisone has been observed, and this may explain the apparent decrease of iodine uptake by the thyroid gland. (36-37)(42)

The response to stress at first appeared to be the result of the action of excess cortical steroids. Negative nitrogen balance was early observed to be one feature of the response to stress. (39-41) It was postulated that the degree of nitrogen excretion was an indication of the level of cortical hormone. (3) However, it has been shown that a minimum amount of cortical hormone is necessary for the response to stress, but that the response is not specifically caused by the increased secretion of cortical hormone. (39) At present, it seems likely that the magnitude of the stress determines the protein catabolic response rather than the amount of adrenal hormone, provided the minimal amount is present. Other factors modify the protein catabolic response to stress. "---there are at least three interdependent variables modifying nitrogen metabolism during stress, namely, the stress itself, the adrenal cortex and the nutritive state of the organism at the time of stress, and each of these must be considered in interpreting experiments on stress and/or adrenal hormone action". (44)

The thyroid gland responds to stress with an increase in activity. (49-51) It has been found that the protein catabolic reponse is greater when both the thyroid hormone and the adrenal cortical hormone are present than when either is present by itself. (17) Several observers believe the mechanisms of action of the thyroid hormone and of the adrenal hormone are independent of one another. (14)(43)

Another observer believes that the evidence points towards the thyroid hormone potentiating the protein catabolic effect of the adrenal steroids. (17) It has been suggested that many of the changes following stress are due to an increase in thyroid activity and that the adrenal cortex is responsible for the reestablishment of homeostasis. (50)

The present experiments were undertaken to further study the peripheral interaction of the thyroid and adrenal hormone.

Materials and Methods

Animals:

The animals used in this experiment were male, albino rats obtained from a local dealer.

Food:

The rats were fed Purina Rat Chow ad libidum prior to thyroidectomy. After thyroidectomy and for the remainder of the experiment the rats were fed Purina Rat Chow to which was added 1% sodium chloride by weight and calcium lactate 0.1gm/15 food.

The chow was dispensed in ointment tins which had a small hole in the top large enough to admit the animal's head. The tins were filled only partially. The tins were in the animal's cages

from about 4:00 P.M. until 9:30 A.M. The tins were weighed before and after feeding.

Water was supplied ad libidum.

Cages:

The rats were housed in individual metabolic cages. These cages were washed daily.

Collection of Urine:

Twenty four hour urine samples were collected at approximately 10:00 A.M. each morning. Toluene was added to the collection bottle as a preservative during the collection period. The samples were frozen soon after collection and remained frozen until processed.

Subsequent experimentation using measure volumes of water showed a recovery of 91% $\pm$ 1.19 from the cages. This was attributed to wetting of the galvanized cage bottoms.

Nitrogen Determination:

Urine from each animal in a group was measured, then pooled, and an aliquot used for nitrogen determination.

The Nesslerization process used is described in "Manual of Standardized Procedures For Spectrophotometric Chemistry". (53) A Klett-Summerson coloremeter was used for the color readings. The standard curve was constructed from readings of ammonium sulphate and urea samples processed by this method.

Operations:

Ether was used as the anesthetic agent. Clean but not sterile technic was used throughout. Silk sutures were used to close the strap muscles of the neck, to appose the flank muscles, and to close the skin.

Autopsy was performed on each animal. Gross thyroid tissue was searched for in both the neck and the anterior mediastinum. Gross adrenal tissue was sought in the region of the kidneys. If either thyroid tissue or adrenal tissue or localized abscess along the suture line were found the animal's data was discarded.

Medications:

Sodium L-thyroxine was dissolved in saline at a pH slightly greater than eight. A stock solution of 6 micrograms/ml. was made. Aliquots of this stock solution were frozen until used. No aliquot was used more than three times nor did the total unfrozen time exceed two hours.

Cortone acetate (Merck) diluted with saline to a concentration of 2.5 mg/ml was used for injection.

All injections were given subcutaneously as Greenspan, Gifford, and Deming (52) found this route superior to intraperitoneal injection.

Stress:

The animals were immersed to a point approximately half the distance between the tip of the nose and the base of the tail in water at 48 C. for two minutes.

Temperature:

The room in which the animals were housed was air conditioned and kept at a constant temperature, 73F.

Experimental

For this experiment the animals were divided into seven groups: (1)shams,(2)untreated, treated-(3) thyroxine 6 micrograms, (4)cortisone 2.5 mg/kg, (5)cortisone 2.5mg/kg & thyroxine 4 micrograms, (6)cortisone 5mg/kg & thyroxine 4 micrograms, and (7) thyroxine 12 micrograms & cortisone 2.5 mg/kg. In each group there were three animals except groups five and seven in which there were only two animals. The dosage of thyroxine approximates a euthyroid level except in group seven where it is hyperthyroid. (10)(54) The dosage of cortisone is physiologic except in group six where it is somewhat increased.

Operations were performed as indicated in figures 1-7. The total length of the experiment for each group was twenty four days.

It is to be pointed out that during the process of being stressed, the rats voided and defecated into the water. Because of the large volume of water and the relatively small amount of urine added to it, the leeching of the feces, and the addition of some hair it was decided that an aliquot of the water would not be analysed for nitrogen. Therefore, one should keep in mind when viewing figures 1-7 that the nitrogen output on day 14 is about 10% less than it should be. Total nitrogen output in this experiment equals the urinary output of nitrogen plus 10% of this as an estimated amount which would be contributed by the stools and hair loss; these latter two determinations were not made.

The results of this experiment can be seen in Figures 1-7 and Table I. More detailed information can be found in Tables II-VIII in the appendix.

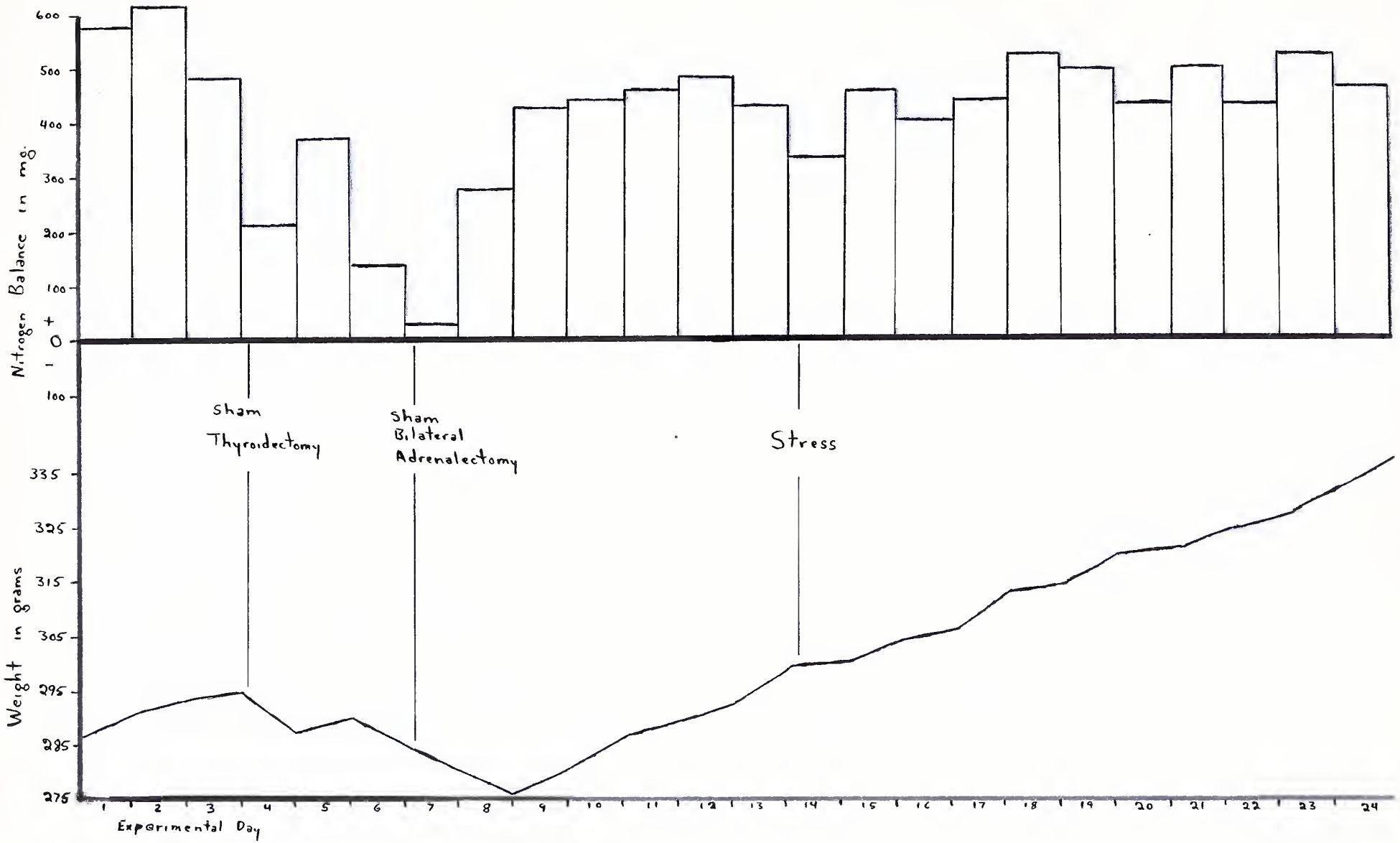

Figure I

Sham Group

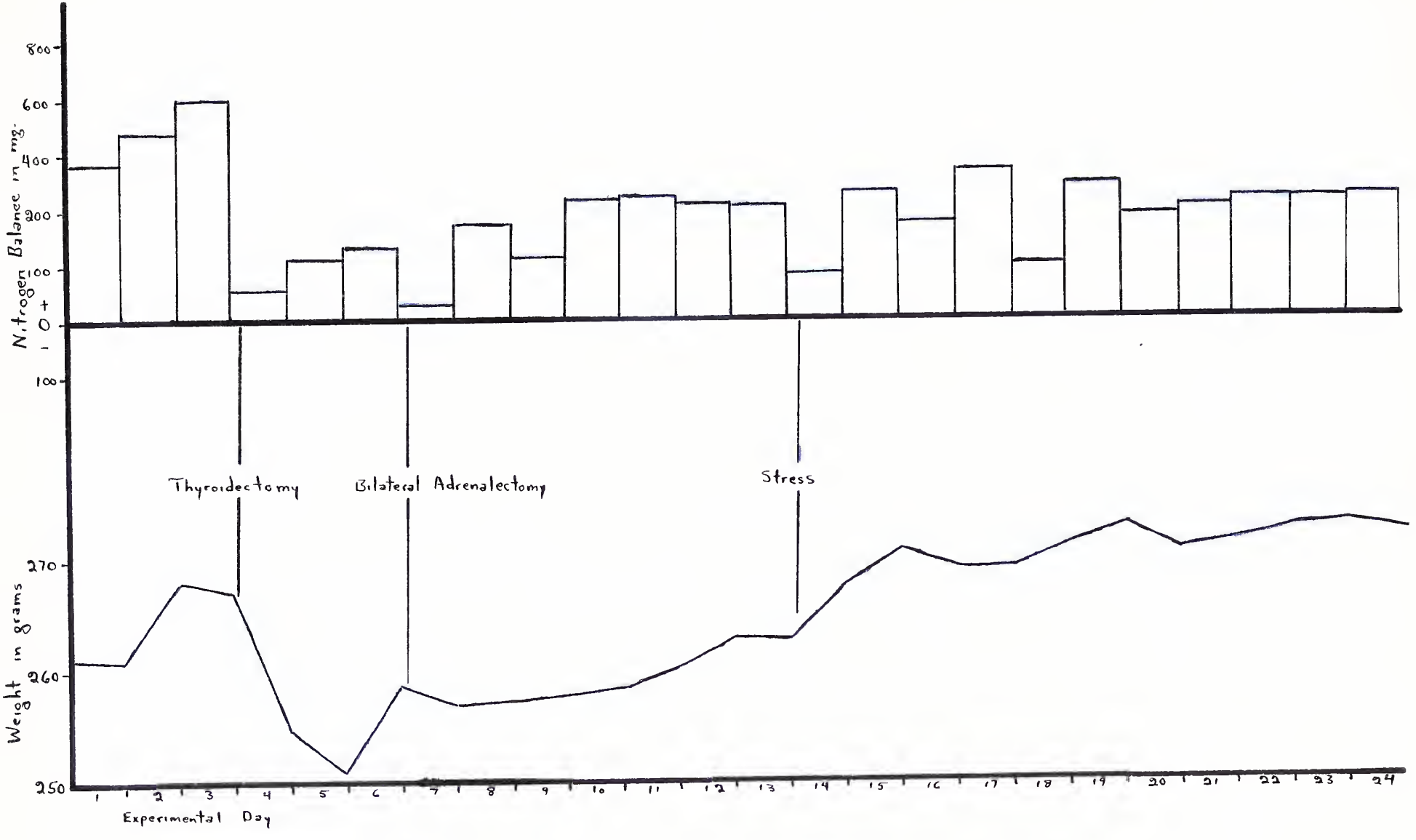

Figure II

Untreated Group

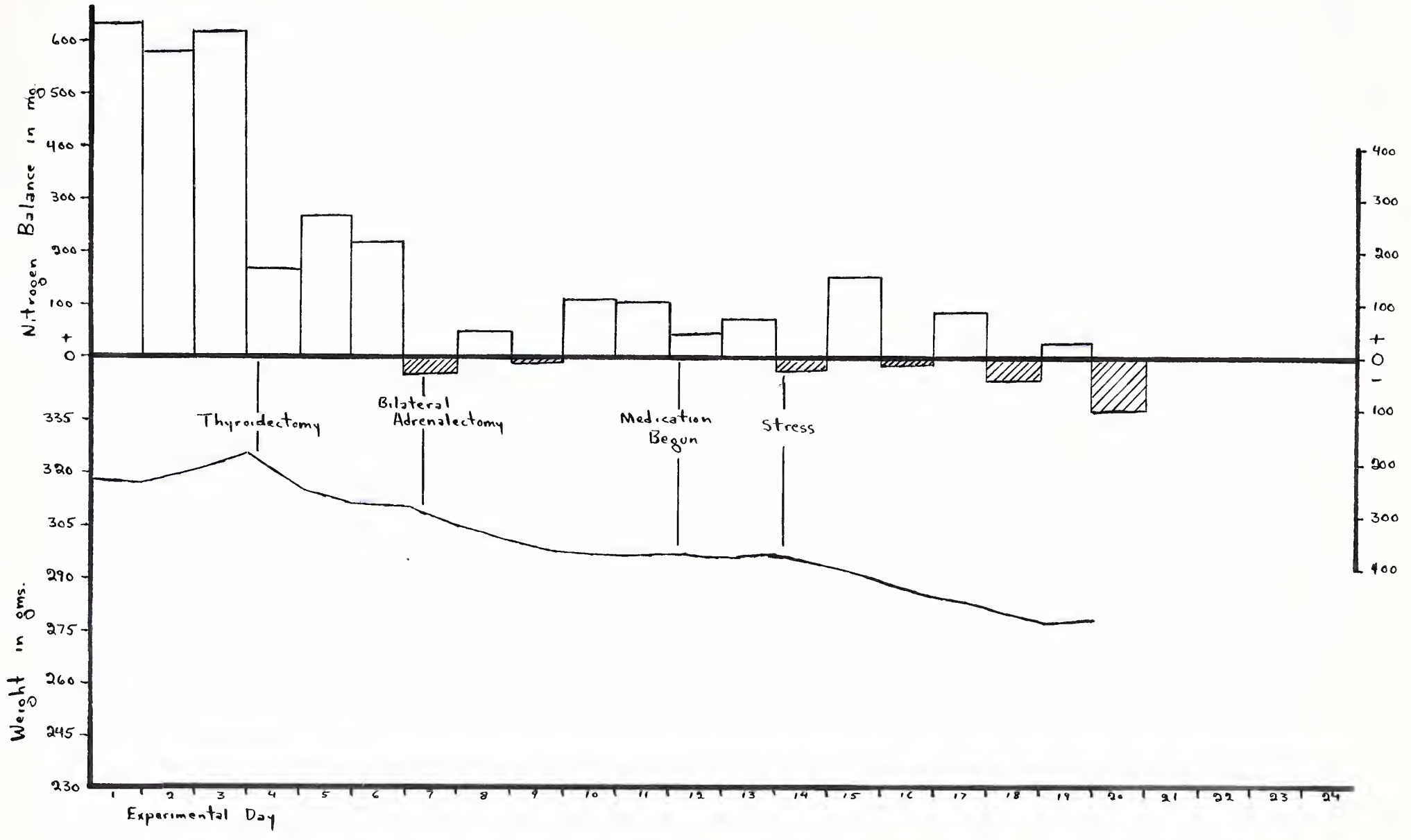

Figure III

L-Thyroxine 6×10^{-6} gm.

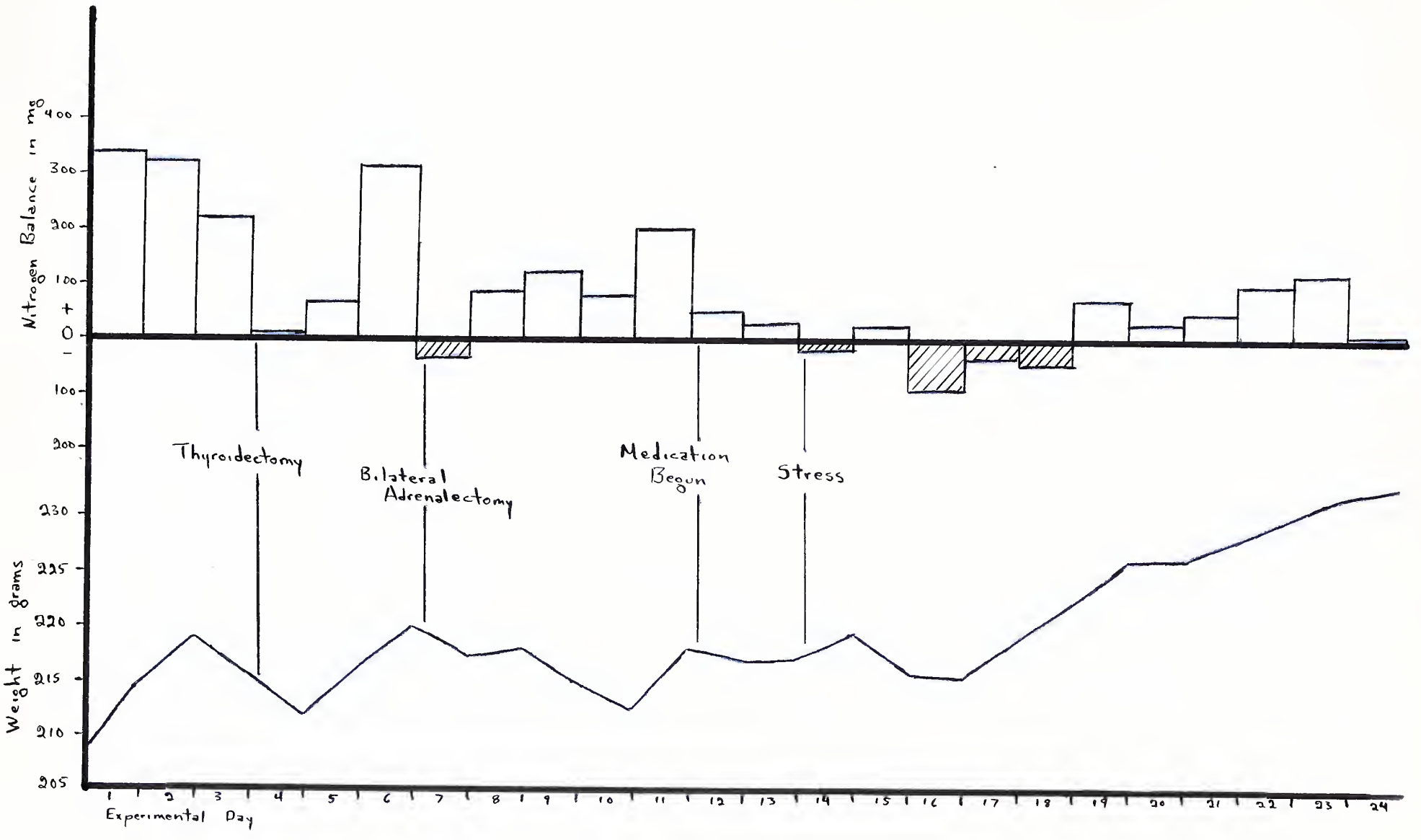

Figure IV

Cortisone 2.5mg./Kg.

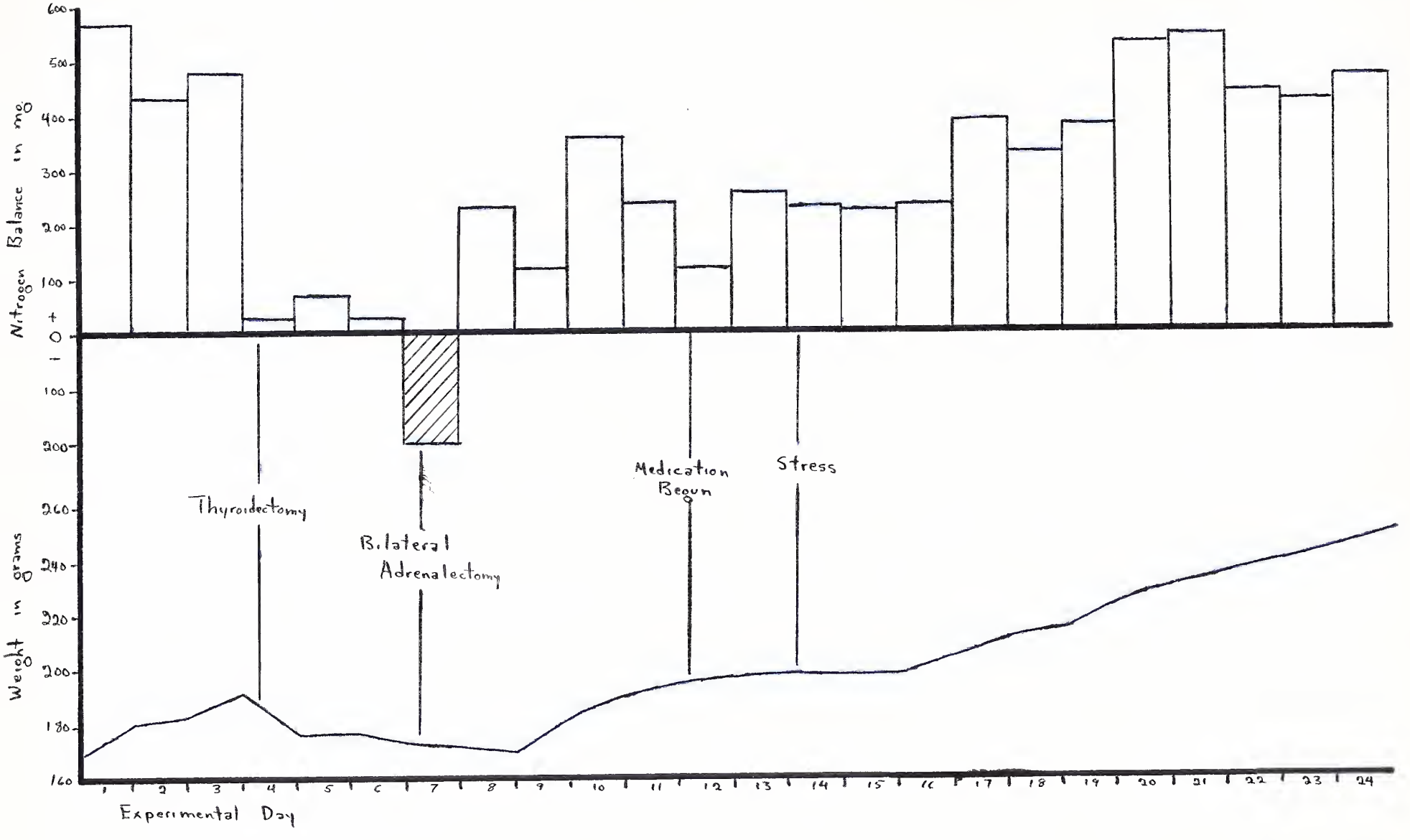

Figure V

L-Thyroxine 4×10^{-6} gm; Cortisone 2.5 mg./Kg.

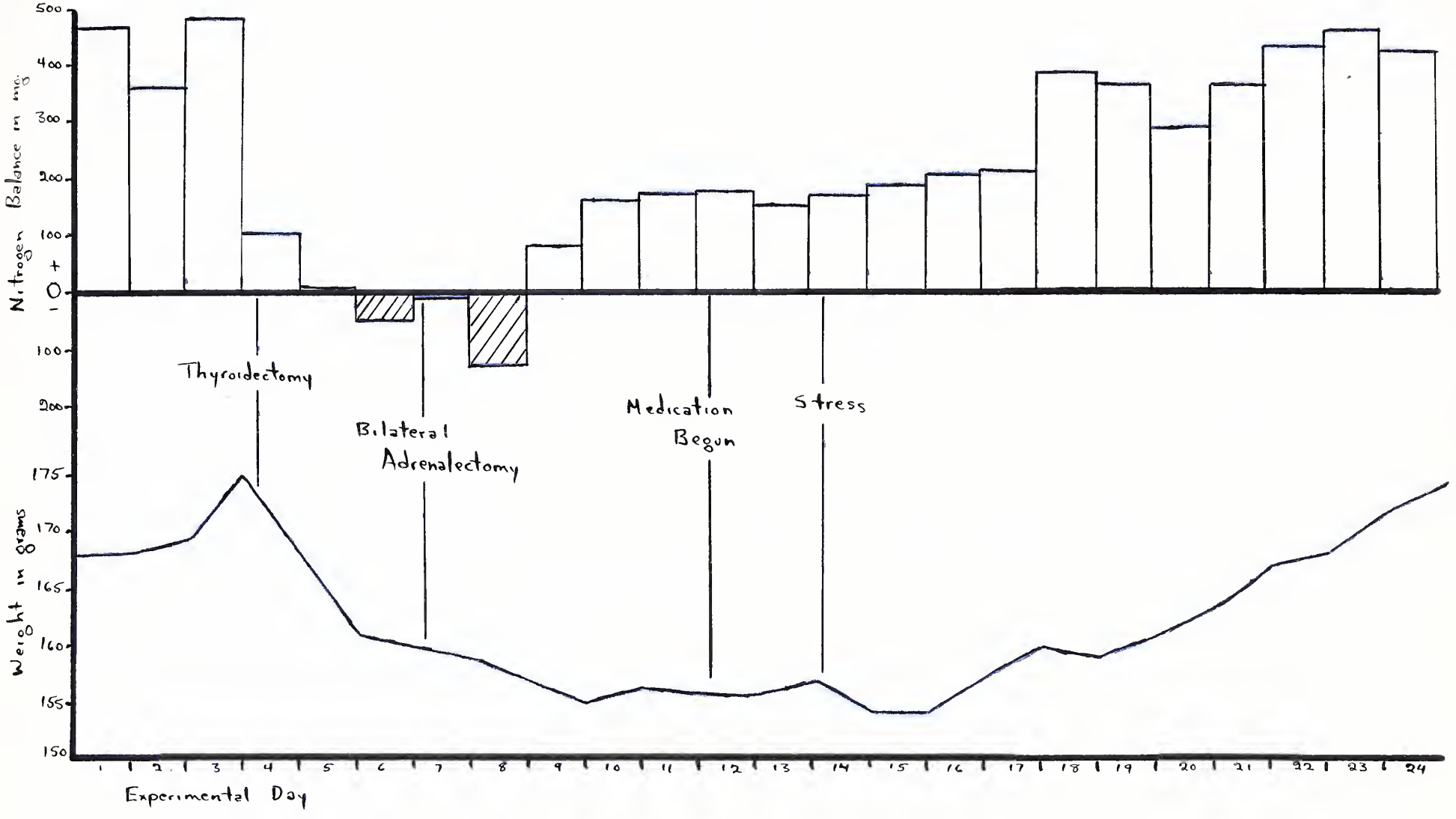

Figure VI

L-Thyroxine 4×10^{-6} gm; Cortisone 5 mg/kg

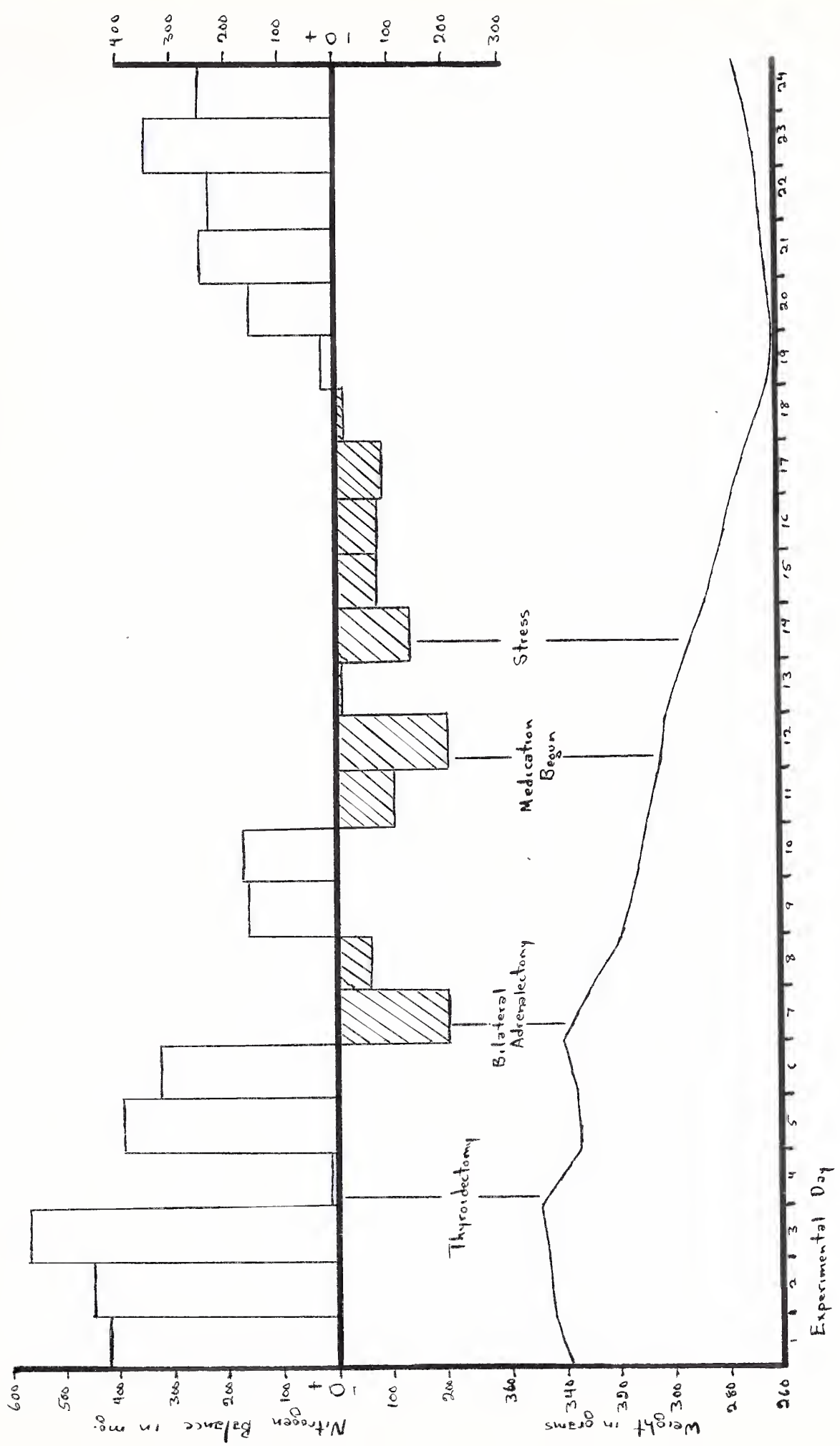

Figure VII

L-Thyroxine 12×10^{-6} gm; Cortisone 2.5 mg/kg

Experimental Day	Sham	Untreated	L-Thyroxine 6×10^{-6} gm	Cortisone 2.5 mg/kg	L-Thyroxine 4×10^{-6} gm Cortisone 2.5 mg/kg	L-Thyroxine 4×10^{-6} gm Cortisone 5 mg/kg	L-Thyroxine 12×10^{-6} gm Cortisone 2.5 mg/kg
1	7.87	8.47	7.22	8.8	11.9	10.2	7.57
2	8.12	9.72	6.78	9.28	9.81	9.02	7.62
3	7.3	9.32	7.00	8.37	10.7	10.84	7.69
4	5.06	5.73	3.86	7.00	2.98	5.65	3.76
5	6.65	4.71	5.08	6.50	4.51	4.32	6.43
6	4.89	6.53	4.40	8.78	4.44	4.86	5.94
7	4.38	3.10	1.62	4.98	2.98	2.78	0.716
8	5.97	4.93	3.4	6.52	7.99	4.97	1.55
9	7.06	5.48	3.63	6.56	7.00	6.32	2.71
10	7.67	6.08	3.82	6.75	9.56	5.94	2.81
11	7.44	6.11	4.21	8.94	7.80	6.42	2.73
12	7.34	6.30	3.92	7.58	6.73	7.87	4.33
13	6.94	6.35	4.82	7.68	8.54	8.61	3.7
14	7.2	6.60	4.24	6.93	7.54	8.06	3.43
15	7.75	6.88	4.48	7.85	7.14	8.96	2.96
16	7.4	6.40	3.64	7.58	8.58	9.96	3.71
17	7.18	7.05	3.96	7.59	9.63	10.12	3.52
18	7.28	5.67	3.34	7.35	9.23	10.02	3.7
19	7.81	6.14	3.19	7.62	9.58	10.5	5.09
20	7.05	5.27		7.47	10.08	8.49	7.32
21	7.34	5.52		7.31	9.50	10.08	8.55
22	7.00	6.06		7.47	9.75	10.35	8.64
23	7.24	6.02		7.45	9.13	10.57	8.65
24	6.9	5.93		6.98	9.37	9.93	8.68

Table I

Food Intake gms./100 gm wt.

Discussion

By using an experimental animal whose thyroid and adrenal glands have been extirpated, the possibility of glandular inhibition has been removed; therefore, observed effects should be due to the peripheral interaction of the two hormones.

A study of figs. I and VI show that the sham group and the thyroxine 4 microgram and cortisone 2.5 mg/kg group responded to stress in qualitatively the same manner. The untreated group reacted as might be expected; nitrogen excretion became rather constant following adrenalectomy. The relative decrease in positive nitrogen balance of the day of stress may be explainable by the large weight gain on that day.

That thyroxine alone increases protein catabolism is in agreement with the observations of Koelsche and Kendall, and Rupp, Paschkis, and Cantarow. Table I shows a decreased food intake by this group in comparision with the sham group. In the light of Engel's observation as to the relationship of nitrogen balance, stress, and the state of nutrition the results of group three must be modified to get a true impression of the role of thyroxine acting by itself.

Cortisone alone also produced a negative nitrogen balance, and this observation is in agreement with that of Hoberman and others. It is interesting to note that while the animals were in a negative nitrogen balance following stress they were gaining weight. The explanation of this is not clear.

A comparision of figs. V and VI shows that increased cortical steroid causes no remarkable response to stress. Although the weight gain of group V post stress is twice that of group VI, there is no such discrepancy in nitrogen balance. This seems to conform to Ingle's concept of the necessity of cortical hormone for a response to stress, but that the response is not a result of the hormone itself.

Fig. VII indicates that excess thyroxine greatly increases protein catabolism following stress. Table I, however, reveals that during the period of negative nitrogen balance the food intake was very slight. It is very significant that when the food intake increased the nitrogen balance became positive. The return to a positive balance, it seems to me, is contrary to the concept that the two hormones act independently. The sequence of events would seem to indicate that the response to stress is not mediated by an outpouring of the thyroid hormone with subsequent return to a homeostatic state affected by increased cortical secretion. The results seem to be more consistent with the concept that the thyroid hormone potentiates the action of the cortical steroids.

Conclusions

In the thyro-parathyroidectomized, bilateral adrenalectomized, stressed rat:

(1) Thyroxine by itself exerts a protein catabolic response.

(2) Cortisone by itself exerts a protein catabolic response.

(3) Thyroxine seems to potentiate the protein catabolic response of cortisone.

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APPENDIX

EXPERIMENTAL DATA

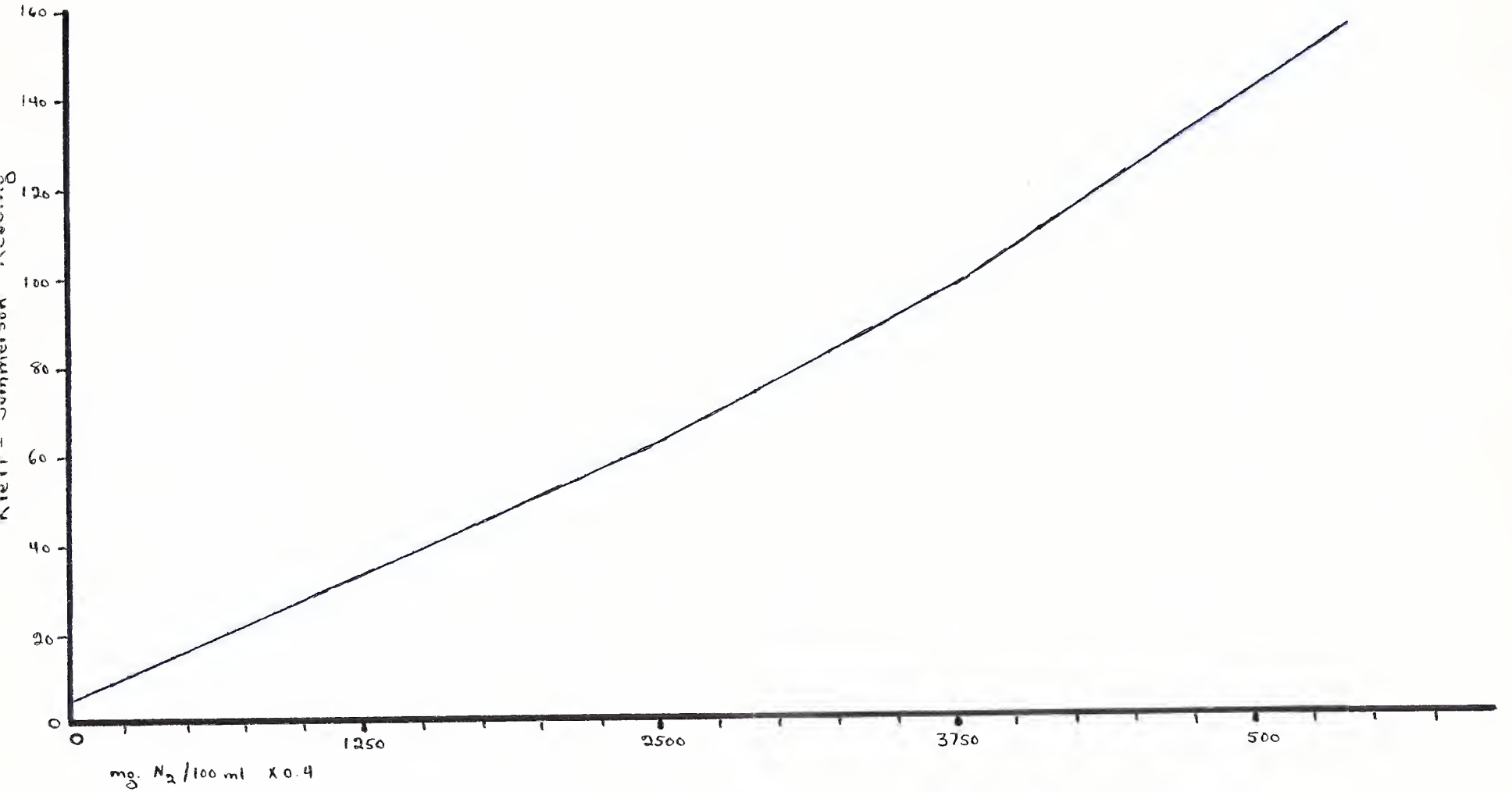


Figure I- Appendix

Urinary Nitrogen Curve

Experimental Day	Animal Weight in grams				Food Intake in grams				Avg. Food N_2 Intake in mg.	Avg. Urine N_2 Output in mg.
	#45	#47	#48	Average	#45	#47	#48	Average		
Start	306.7	257.2	294.6	286.2						
1	307.5	265.6	297.9	290.3	25.2	21.1	21.4	22.6	845	243
2	312.4	268.4	298.5	293.1	24.8	20.9	25.2	23.63	883	243
3	313.7	269.4	300.7	294.6	23.4	20.7	20.1	21.4	800	289
4 ●	314.8	263.3	283.4	287.2	19.1	12.7	12.3	14.7	541	300
5	315.6	265.6	288.7	290.0	22.3	18.8	16.5	19.2	707	308
6	320.8	251.0	282	284.6	25.6	3.1	13.4	14.03	516	345
7 ▬	313.5	250.7	272.9	279.0	15.8	12.0	9.2	12.3	453	385
8	317.3	250 3	258.4	275.3	30.2	16.6	3.5	16.6	611	304
9	317.4	261.1	264.3	286.9	28.9	19.9	10.1	19.6	724	270
10	319.2	265.2	275.6	286.5	27.8	20.4	17.6	21.7	800	328
11	323.9	268.2	275.7	289.2	29.1	21.8	13.3	21.4	788	303
12	323	271.5	282.7	292.4	23.5	21.4	20.5	21.4	788	277
13	327.2	278.7	291.6	299.2	21.1	21.9	18.4	20.36	755	290
14 ◆	325.8	280.9	293.6	300.1	24.5	21.7	21.3	21.5	791	415
15	328.3	285.8	299.3	304.5	24.6	22.8	23.1	23.3	858	367
16	331.0	289.4	298.8	306.4	24.2	23.4	21.3	22.63	835	395
17	335.3	293.4	310.6	313.1	22.5	22.4	21.8	22.23	818	341
18	336.4	296.7	310.7	314.6	24.0	23.5	20.9	25.8	840	288
19	342.3	304.6	313.9	320.3	28.5	24.4	21.5	24.8	913	376
20	345.5	306.4	310.4	320.8	27.2	21.1	19.5	22.6	830	357
21	347.8	308.4	317.0	324.4	23.3	24.1	24.2	23.86	880	346
22	352.8	310.1	317.9	326.9	25.7	23.5	19.1	22.76	839	368
23	355	315.6	326.4	332.3	23.7	26	21.9	23.86	880	327
24	358.9	323.9	327.8	336.9	25.6	23.5	20.1	23.06	847	349

● Day of Sham Thyroidectomy
▬ Day of Sham Bilateral Adrenalectomy
◆ Day of Stress

Table II-A

Sham Group

Estimated Hair + Fecal N_2 in mg.	Total N_2 Output in mg.	Nitrogen Balance in mg.		Urine Volume in ml.				Dilution Factor	Klett-Summerson Readings			N_2 in mg/ml of urine as diluted
		Positive	Negative	#45	#47	#48	Average		1	2	Average	
24.3	267.3	577.7		5.8	7.5	8.5	7.27	2	113	111	112	16.7
24.3	267.3	616.7		6.2	7.4	9.9	7.83	2	102	102	102	15.5
28.9	317.9	482.1		6.6	8.1	10.5	8.4	2	115	117	116	17.2
30.0	330.0	211.0		10.7	8.8	17.5	12.3	2	77	78	77.5	12.2
30.8	338.8	368.2		7.7	10.4	18.3	12.12	2	80	82	81	12.7
34.5	379.5	136.5		11.2	13.6	24.6	16.45	2	64.5	64.5	64.5	10.5
38.5	493.5	29.5		17.7	16.0	38.4	24.03	2	50	50	50	8.0
30.4	334.4	276.6		11.4	15.9	21.4	16.32	2	58.5	56.5	57.5	9.3
27.0	297.0	427.0		9.0	12.9	16.4	12.75	2	65	66	65.5	10.6
32.8	360.8	439.2		11.9	10.8	17.8	13.47	2	79	77	78	12.2
50.3	333.3	454.7		8.8	14.7	17.0	13.47	2	71	70	70.5	11.24
27.7	304.7	483.3		11.8	11.0	14.3	12.35	2	71	69	70	11.2
29.0	319.0	436.0		10.1	10.5	14.6	11.7	2	79	79	79	12.4
41.5	456.5	334.5		16.4	17.9	20.9	18.4	2	71.5	70.5	71	11.25
36.7	403.7	454.3		10.6	11.9	16.4	12.9	2	90	92	91	14.2
39.5	434.5	400.5		11.8	11.0	14.7	12.5	2	104	104	104	15.8
34.1	375.1	442.9		11.6	13.7	15.9	13.73	2	79	79	79	12.4
28.8	316.8	523.2		13.0	12.0	14.7	13.22	2	71	73	72	11.4
37.6	413.6	499.4		11.7	9.7	11.7	11.2	2	105	115	115	16.8
35.7	392.7	437.3		9.5	11.6	14.8	11.9	2	97	97	97	14.99
34.6	380.6	499.4		8.5	9.9	17.9	12.08	2	92	92	92	14.3
36.8	404.8	434.2		12.2	10.3	13.4	11.9	2	100	102	101	15.45
32.7	359.7	520.3		11.3	8.1	12.2	10.52	2	104	102	103	15.52
34.9	383.9	463.1		11.3	8.2	13.0	10.83	2	108	106	107	16.1

Table II-B

Sham Group

Experimental Day	Animal Weight in grams				Food Intake in grams				Avg. Food N_2 Intake in mg.	Avg. Urine N_2 Output in mg.
	# 30	# 33	# 36	Average	# 30	# 33	# 36	Average		
Start	274.0	208.0	301.4	261.1						
1	275.4	208.6	298.5	260.8	25.5	15.1	25.8	22.13	834	417
2	290.9	212.6	300.5	268.0	34.9	16.8	25.3	25.66	962	447
3	284.5	217.4	299.2	267.0	29.7	19.3	25.6	24.86	933	317
4 ●	289.5	196.9	275.6	254.7	21.2	9.4	3.2	14.6	538	441
5	279.4	216.6	255.9	250.6	15.6	19.8	0.3	11.9	438	285
6	302.7	217.6	256.1	258.8	25.9	19.6	4.3	16.6	612	413
7 ■	302.9	215.3	251.8	256.7	13.7	8.1	2.2	8.0	294	243
8	301.3	210.6	259.4	257.1	18.1	11.0	8.9	12.66	468	275
9	300.9	213.4	259.1	257.8	19.1	11.5	12.0	14.2	523	377
10	304.4	214.5	256.4	258.4	19.9	13.2	14.0	15.7	575	311
11	308.4	216.2	255.1	259.9	19.3	12.3	14.9	15.83	583	313
12	305.7	218.4	263.9	262.7	17.9	12.7	18.6	16.4	604	356
13	303.8	223.0	268.6	265.1	18.2	14.9	18.1	16.73	617	374
14 ◆	308.3	223.4	270.2	267.6	19.1	14.3	19.3	17.56	647	513
15	305.7	232.0	273.8	270.5	18.3	19.2	17.8	18.43	679	386
16	304.1	228.5	274.4	269.0	17.7	16.8	17.2	17.23	635	420
17	306.2	226.3	274.6	269.0	24.3	15.0	16.6	18.96	700	341
18	309.0	226.4	277.6	271.0	16.9	11.7	17.4	15.33	565	425
19	308.7	229.9	280.3	273.0	17.8	14.2	18.1	16.7	615	310
20	298.4	233.1	280.3	270.6	10.1	14.9	17.9	14.3	527	310
21	299.4	234.1	280.1	271.2	12.6	13.5	18.8	14.96	552	314
22	300.0	237.5	279.6	272.4	16.0	16.5	16.8	16.43	606	338
23	296.4	239.6	282.4	272.8	16.5	14.2	18.5	16.4	604	345
24	293.3	239.6	282.9	271.9	16.1	16.2	15.8	16.03	591	322

● Day of Thyroidectomy
■ Day of Bilateral Adrenalectomy
◆ Day of Stress

Table III-A

Untreated Group

Estimated Hair & Fecal N_2 in mg.	Total N_2 Output in mg.	Nitrogen Balance in mg.		Urine Volume in ml.				Dilution Factor	Klett - Summerson Readings			N_2 in mg/ml of urine as diluted
		Positive	Negative	#30	#33	#36	Average		1	2	Average	
41.7	458.7	375.3		13.7	10.0	13.6	12.4	2	114	112	113	16.8
44.7	491.7	470.3		21.1	9.5	13.0	14.5	2	100.5	101	100.75	15.4
31.7	348.7	584.3		4.8	11.0	10.4	8.73	2	126	124	125	18.2
44.1	485.1	52.9		21.6	9.7	25.8	19.0	2	73	73	73	11.6
28.5	313.5	124.5		2.9	13.4	22.0	12.76	2	68	70	69	11.0
41.3	454.3	157.7		11.6	9.0	25.6	15.4	2	84.75	86	85.87	13.4
24.3	267.3	26.7		18.0	18.1	34.2	23.4	2	33	35	34	5.2
27.5	302.5	166.5		20.0	12.3	10.2	14.17	2	59	61	60	9.72
37.7	414.7	110.3		21.0	14.8	22.5	19.4	2	59	61	60	9.72
31.1	342.1	232.9		19.4	10.9	29.5	19.9	2	48	50	49	7.8
31.3	344.3	238.7		16.3	10.6	27.4	18.1	2	53	55	54	8.65
35.6	391.6	212.4		20.2	11.8	30.6	20.7	2	55	53	54	8.65
37.4	411.4	205.6		18.3	11.2	31.1	20.2	2	59	57	58	9.35
51.3	564.3	89.7		27.6	14.9	29.4	23.9	2	67	67	67	10.7
38.6	424.6	254.4		12.4	12.8	25.6	16.9	2	72	72	72	11.4
420	462.0	173.0		12.8	12.3	28.4	17.8	2	75	75	75	11.8
34.1	374.1	325.9		12.6	7.4	29.8	16.3	2	66.5	64.5	65.5	10.5
42.5	467.5	97.5		17.1	9.7	23.8	16.7	2	81	81	81	12.7
31.0	341.0	274.0		14.3	9.4	26.2	16.6	2	57	59	58	9.35
31.0	341.0	186.0		13.0	10.8	29.2	17.6	2	54	56	55	8.8
31.4	345.4	206.4		16.1	10.1	24.1	16.8	2	58	58	58	9.35
33.8	371.8	234.2		13.8	11.5	24.7	16.6	2	67	65	66	10.5
34.5	379.5	224.5		18.7	9.4	23.7	17.3	2	62	62	62	10.0
32.2	354.2	236.8		16.5	12.7	17.7	15.6	2	63	63	63	10.3

Table III-B

Untreated Group

Experimental Day	Animal Weight in grams				Food Intake in grams				Avg. Food N_2 Intake in mg.	Avg. Urine N_2 Output in mg.
	# 44	# 69	# 72	Average	#44	# 69	# 72	Average		
Start	249.3	323	380.4	317.6						
1	254.8	321.3	371.9	316.0	22.9	24	22	22.97	858	206
2	258.5	324.3	376.6	319.8	24.4	17.9	22.3	21.53	805	207
3	266.7	328.9	381.6	325.7	18.8	23.6	24.3	22.6	846	210
4 ●	259.1	321.4	365.4	315.3	13.0	11.7	12.4	12.36	456	265
5	239.2	323.3	371.2	311.2	4.8	27.1	15.8	15.9	586	290
6	241.6	320.4	368.9	310.3	9.3	14.5	16.9	13.66	504	260
7 ■	242.7	310.6	361.2	304.8	6.2	4.8	3.9	4.97	183	196
8	240.4	308.5	352.3	300.4	11.4	9.5	10.1	10.3	379	302
9	236.6	302.9	351.2	296.9	10.9	9.1	12.6	10.87	400	367
10	232.4	304.6	351.9	296.3	10.3	13.5	10.2	11.33	418	287
11	234.7	305.4	347.8	296.0	12.2	13.1	12.1	12.46	459	326
12 ▲	240.3	303.4	342.0	295.2	14.0	13.1	7.6	11.57	426	349
13	241.4	304.4	344.2	296.6	12.0	16.5	14.3	14.27	526	415
14 ◆	238.9	299.1	331.2	293.1	15.9	12.4	9.2	12.5	460	443
15	238.6	294.4	330.7	287.9	15.6	9.6	13.9	13.03	480	368
16	236.0	284.4	329.5	283.3	12.4	7.1	11.7	10.4	383	336
17	234.6	278.9	329.2	280.9	13.7	5.5	14.3	11.17	412	298
18	234.9	269.7	328.9	277.8	13.2	1.6	15.0	9.93	366	371
19	233.8	265.0	335.2	278.0	9.6	0.5	16.5	8.87	326	274
20		+								
21										
22										
23										
24										

● Day of Thyroidectomy
■ Day of Bilateral Adrenalectomy
+ Death of Experimental Animal

▲ Day Medication Began
◆ Day of Stress

Table IV-A

Thyroxine 6×10^{-6} gm.

Estimated Hair & Fecal N_2 in mg.	Total N_2 Output in mg.	Nitrogen Balance in mg.		Urine Volumes in ml.				Dilution Factor	Klett – Summerson Readings			N_2 in mg/ml of urine as diluted
		Positive	Negative	# 44	# 69	# 72	Average		1	2	Average	
20.6	226.6	631.4		7.9	4.6	2.7	5.06	2	144	144	144	20.4
20.7	227.7	577.3		8.2	5.1	3.3	5.53	2	128	130	129	18.7
21.0	231.0	615		10.6	6.1	2.6	6.43	2	109	109	109	16.4
26.5	291.5	164.5		12.1	5.6	6.1	7.93	2	112	112	112	16.7
29.0	319.0	276		34.0	6.6	6.6	15.73	2	56	58	57	9.2
26.0	286.0	218		20.8	8.7	8.6	13.33	2	60	60	60	9.8
19.6	215.6		32.6	39.5	36.2	31.7	35.8	2	26	26	26	3.8
30.2	332.2	46.8		22.7	17.5	17.9	19.36	2	50	48	49	7.8
36.7	403.7		3.7	27.5	18.9	23.3	23.23	2	44	44	44	6.9
28.7	315.7	102.7		29.0	16.1	15.5	20.2	2	45	45	45	7.1
32.6	358.6	100.4		24.3	17.9	15.8	19.66	2	53	52	52.5	8.3
34.9	383.9	42.1		26.0	17.5	16.0	19.83	2	55	55	55	8.8
41.5	456.5	69.5		23.1	15.2	22.0	20.1	2	65	64	64.5	10.3
44.3	487.3		27.3	27.3	15.5	25.5	22.76	2	60	60	60	9.73
30.0	330.0	150		24.4	12.8	20.4	19.2	2	49	49	49	7.8
33.6	369.6	13.4		21.0	11.4	16.6	16.33	2	64	64	64	10.29
29.8	327.8	84.2		14.3	11.0	19.7	16.66	2	56	55	55.5	8.91
37.1	408.1		42.1	15.8	7.7	37.5	20.33	2	58	56	57	9.13
27.4	301.4	24.6		14.3	4.7	20.1	13.03	2	66	66	66	10.5

Table IV-B

Thyroxine 6×10^{-6} gm

Experimental Day	Animal Weight in grams				Food Intake in grams				Avg. Food N_2 Intake in mg.	Avg. Urine N_2 Output in mg.
	# 21	# 22	# 29	Average	# 21	# 22	# 29	Average		
Start	213.3	178.2	233.4	208.3						
1	216.9	189.9	236.0	214.3	15.8	18.1	21.7	18.53	694	323
2	219.4	196.4	240.2	218.7	16.3	22.1	21.9	20.1	753	391
3	230.9	179.0	245.1	215.0	16.3	14.2	23.9	18.13	679	416
4 ●	213.9	192.2	228.8	211.6	12.2	22.0	16.5	14.9	549	495
5	217.2	201.6	230.4	216.4	5.7	23.4	12.6	13.9	513	395
6	214.1	208.3	237.4	219.9	8.8	20.4	18.2	19.13	705	351
7 ■	213.4	202.1	236.7	217.4	6.8	11.4	14.5	10.9	402	398
8	209.3	204.1	239.4	217.6	10.4	15.6	16.5	14.17	523	399
9	202.4	205.9	236.4	214.6	7.0	17.5	18.0	14.17	523	364
10	201.5	202.8	232.4	212.2	8.4	18.6	15.1	14.37	529	408
11	199.7	209.9	243.4	217.7	23.9	15.2	18.6	19.23	709	458
12 ▲	205.4	207.6	247.3	216.7	15.4	14.5	19.4	16.43	606	504
13	204.5	206.2	246.4	217.0	16.1	15.7	18.1	16.63	613	521
14 ◆	206.9	204.0	246.9	219.3	11.8	15.6	17.9	15.1	557	580
15	199.4	199.6	248.4	215.8	16.7	16.6	17.9	17.07	629	545
16	199.6	199.4	247.2	215.4	15.6	14.4	19.0	16.3	601	628
17	203.9	202.4	249.3	218.9	16.6	14.2	18.6	16.47	607	580
18	204.1	213.9	249.3	222.4	15.7	13.7	19.2	16.2	597	579
19	214.4	208.9	255.6	226.3	17.2	15.4	18.7	17.1	630	505
20	215.9	206.0	256.6	226.2	18.6	15.1	18.9	16.87	622	537
21	217.3	206.5	260.5	228.1	16.4	14.1	19.3	16.6	612	511
22	220.4	208.4	262.3	230.4	17.8	14.3	19.2	17.1	630	477
23	222.3	208.9	263.4	231.5	17.3	15.1	19.2	17.2	634	465
24	222.4	208.4	266.6	232.5	17.0	13.8	17.8	16.2	597	543

● Day of Thyroidectomy
■ Day of Bilateral Adrenalectomy
▲ Day Medication Begun
◆ Day of Stress

Table V-A

Cortisone 2.5mg/kg.

Estimated Hair + Fecal N_2 in mg.	Total N_2 Output in mg.	Nitrogen Balance in mg.		Urine Volume in ml.				Dilution Factor	Klett - Summerson Readings			N_2 in mg/ml of urine as diluted
		Positive	Negative	# 21	# 22	# 29	Average		1	2	Average	
32.3	355.3	338.7		8.3	8.0	7.7	8.0	2	143	141	142	20.2
39.1	430.1	322.9		8.6	12.1	8.5	9.73	2	140	142	141	20.1
41.6	457.6	221.4		9.5	15.8	7.7	11.0	2	135	133	134	18.8
49.5	544.5	4.5		11.2	20.2	33.2	21.53	2	72	73	72.5	11.48
39.5	434.5	68.5		11.5	17.8	18.5	15.93	2	80	78	79	12.4
35.1	386.1	318.9		9.9	13.7	15.6	13.06	2	87	87	87	13.5
39.8	437.8		35.8	17.1	16.3	19.5	17.63	2	71	71	71	11.3
39.9	438.9	84.1		17.8	14.9	23.7	18.8	2	65	67	66	10.6
36.4	400.4	122.6		11.0	15.7	22.5	16.4	2	70	70	70	11.1
40.8	448.8	80.6		37.5	30.3	28.5	28.77	2	46	46	46	7.22
45.8	503.8	205.2		31.2	31.5	23.9	28.86	2	49.5	49.5	49.5	7.93
50.4	554.4	51.6		30.1	17.5	25.7	24.43	2	64.0	64	64	10.3
52.1	573.1	29.9		28.6	21.2	25.2	25.0	2	65	65	65	10.43
52.0	572.0		15	24.1	23.5	25.9	24.5	2	66	66	66	10.6
54.5	599.5	29.5		24.6	20.6	28.5	24.56	2	70	70	70	11.1
62.8	696.8		89.8	34.6	21.9	27.4	27.96	2	70.8	70.8	70.8	11.23
58.0	638.0		31.0	33.0	22.5	26.4	27.3	2	66	66	66	10.6
57.9	636.9		39.9	31.1	27.4	23.3	27.26	2	67	65	66	10.6
50.5	555.5	74.5		27.9	24.8	21.0	24.56	2	65.5	63.5	64.5	10.3
53.7	590.7	31.3		21.6	24.8	22.3	22.9	2	73	73	73	11.52
51.1	562.1	499		21.1	26.6	19.9	22.53	2	71	72	71.5	11.35
47.7	524.7	105.3		20.5	23.3	21.7	21.83	2	68.3	68.3	68.3	10.9
46.5	511.5	122.5		26.5	26.5	22.6	25.2	2	58	59	58.5	9.2
54.3	597.3		0.3	22.9	22.7	22.2	22.6	2	77	75	76	12.0

Table V-B

Cortisone 2.5mg/Kg.

Experimental Day	Animal Weight in grams			Food Intake in grams			Avg. Food N_2 Intake in mg.	Avg. Urine N_2 Output in mg.	Estimated Hair & Fecal N_2 in mg.	Total N_2 Output in mg.
	#60	#61	Average	#60	#61	Average				
Start	178.4	160.8	169.6							
1	182.8	178.1	180.45	21.0	20.8	20.9	782	198	19.8	217.8
2	185.4	181.9	183.7	18.5	17.2	17.85	668	216	21.6	237.6
3	193.0	189.9	191.95	20.4	19.8	20.1	750	248	24.8	272.8
4 ●	180.6	172.4	176.5	6.6	4.6	5.6	256	213	21.3	234.3
5	182.0	173.4	177.7	9.0	7.0	8.0	294	212	21.2	233.2
6	163.4	184.4	173.9	0.1	15.5	7.8	286	236	23.6	259.6
7 ■	172.3	172.4	172.35	5.7	4.6	5.15	189	358	35.8	393.8
8	177.4	162.4	169.9	11.3	16.0	13.65	501	249	24.9	373.9
9	182.3	184.3	183.3	11.9	12.8	12.35	458	311	31.1	342.1
10	189.6	190.1	189.85	13.7	22.0	17.85	655	270	27.0	297
11	195.0	192.9	193.95	14.5	15.4	14.95	548	288	28.8	316.8
12 ▲	198.2	195.3	196.75	15.9	10.6	13.15	482	335	33.5	368.5
13	199.4	198.0	198.7	16.3	17.4	16.85	618	332	33.2	365.2
14 ◆	205.4	191.2	198.3	18.0	11.9	14.95	548	291	29.1	320.1
15	211.3	185.4	198.35	18.3	10.0	14.15	520	271	27.1	298.1
16	213.4	193.9	203.65	17.9	16.6	17.25	633	367	36.7	403.7
17	223.4	199.6	211.5	20.3	19.6	19.95	732	317	31.7	348.7
18	227.2	202.3	214.75	20.6	18.7	19.65	721	450	45.0	495
19	232.9	214.8	223.85	21.2	20.9	21.05	735	326	32.6	358.6
20	240.4	219.6	230.0	22.8	23.0	22.9	842	284	28.4	312.4
21	246.6	223.7	235.15	22.6	21.7	22.15	812	242	24.2	268.2
22	247.9	229.9	238.9	22.8	23.5	23.15	850	376	37.6	413.6
23	251.6	234.9	243.25	21.2	22.8	22.0	808	351	35.1	386.1
24	257.6	241.5	249.55	22.4	23.8	23.1	848	356	35.0	385

● Day of Thyroidectomy
■ Day of Bilateral Adrenalectomy
▲ Day Medication Began
◆ Day of Stress

Table VI - A

Thyroxine 4×10^{-6} gm.
Cortisone 2.5 mg/Kg

Experimental Day	Nitrogen Balance in mg.		Urine Volume in ml.			Dilution Factor	Klett - Summerson Readings			N_2 in mg/ml of urine as diluted
	Positive	Negative	#60	#61	Average		1	2	Average	
Start										
1	564.2		4.7	4.9	4.8	2	146	146	146	20.6
2	430.4		5.0	6.3	5.65	2	136	134	135	19.4
3	477.2		6.8	6.4	6.6	2	129	128	128.5	18.8
4	21.7		8.5	10.2	9.35	2	71	71	71	11.4
5	60.8		3.3	13.5	8.4	2	80	80	80	12.6
6	26.4		4.8	15.2	10.0	2	74	74	74	11.8
7		202.8	30.5	48.8	39.4	2	30	30	30	4.54
8	227.1		11.8	38	24.9	2	33	33	33	5.0
9	115.9		12.9	43.6	28.25	2	36	36	36	5.5
10	358		11.3	36.4	23.56	2	37	38	37.5	5.75
11	231.2		12.2	32.8	22.5	2	42	40	41	6.4
12	113.5		12.2	27.2	19.7	2	53	54	53.5	8.5
13	252.8		13.6	22.8	18.2	2	56	57	56.5	9.1
14	227.9		16.9	32.1	24.5	2	39	37	38	5.93
15	221.9		7.5	30.0	18.75	2	46	46	46	7.2
16	229.3		10.3	29.6	19.95	2	58	57	57.5	9.2
17	383.3		11.3	28.4	19.35	2	52	52	52	8.2
18	326		11.4	21.5	16.45	2	88	89	88.5	13.7
19	376.4		11.3	19.4	15.35	2	67	67	67	10.63
20	529.6		15.0	18.5	16.75	2	53	53	53	8.48
21	543.8		13.7	14.8	14.25	2	54	54	54	8.5
22	436.4		13.5	17.9	15.7	2	76	76	76	12.0
23	421.9		11.0	18.3	14.65	2	76	76	76	12.0
24	463		14.3	13.2	13.75	2	84	85	84.5	13.2

Table VI - B

Thyroxine 4×10^{-6} gm

Cortisone 2.5 mg/Kg

Experimental Day	Animal Weight in grams				Food Intake in grams				Avg. Food N_2 Intake in mg	Avg. Urine N_2 Output in mg.
	# 64	# 66	# 67	Average	# 64	# 66	# 67	Average		
Start	154.4	173.9	160 0	162.76						
1	148.4	176.7	163.4	162.83	14.6	35.3 combined		16.63	623	142
2	156.6	182.1	165.9	168.2	17.3	17.4	10.0	14.9	558	188
3	160.3	189.9	173.3	174.5	16.3	19.7	19.9	18.63	698	194
4 ●	162.3	177.6	164.0	167.96	14.9	5.5	8.7	9.7	357	228
5	166.4	171.1	145.4	160.96	15.2	6.1	0.0	7.1	261	237
6	172.4	154.6	153.4	160.13	16.8	0.3	6.3	7.8	287	301
7 ▬	170.4	156.6	149.6	158.86	9.2	1.2	2.9	4.43	163	154
8	168.4	152.4	149.6	156.8	12.1	5.2	6.2	7.83	288	373
9	167.9	148.7	147.9	154.83	12.3	8.5	8.7	9.83	362	252
10	167.4	154.4	146.5	156.1	11.1	9.6	7.0	9.23	340	161
11	167.4	156.4	142.9	155.56	12.9	11 0	6.1	10.0	368	175
12 ▲	169.5	161.9	135.6	155.63	12.7	14.4	9.6	12.23	451	249
13	166.6	166.4	137.2	156.73	14	15.7	10.6	13.43	495	310
14 ◆	168.4	162.9	136.8	154.03	13.1	12.2	12.2	12.5	461	270
15	158.9	163.8	140.3	154.3	14.3	12.8	14.3	13.8	513	292
16	160.1	167.2	144.3	157.2	13.9	16.4	16.2	15.5	571	328
17	160.4	171.4	145.5	159.73	15.7	17.5	15.0	16.06	592	342
18	159.5	171.9	144.9	158.76	15.2	17.8	14.9	15.96	589	182.5
19	159.5	175.4	147.4	160.76	15.6	18.0	16.9	16.83	620	209
20	163.3	179.5	145.9	162.9	14.3	15.1	17.8	13.73	506	194
21	161.0	186.9	153.0	166.93	15.6	15.6	18.7	16 63	614	223
22	160 6	185.4	158.4	168.13	14.1	17.7	20.2	17.33	640	188
23	163.3	191.4	160.2	171 63	15.5	20.9	17.5	17.96	662	182
24	162.9	199.9	159.5	174.1	15.7	20.0	15.7	17.13	632	186

● Day of Thyroidectomy
▬ Day of Bilateral Adrenalectomy
▲ Day Medication Begun
◆ Day of Stress

Table VII-A

Thyroxine 4×10^{-6} gm
Cortisone 5mg/Kg.

Estimated Fecal + Hair N_2 in mg	Total N_2 Output in mg	Nitrogen Balance in mg.		Urine Volume in ml.				Dilution Factor	Klett-Summerson Readings			N_2 in mg/ml of Urine as diluted
		Positive	Negative	#64	#66	#67	Average		1	2	Average	
14.2	156.2	466.8		2.5	5.5	4.5	4.16	2	115	115	115	17.05
18.0	198.0	360		5.0	4.5	3.9	4.46	2	142	142	142	20.2
19.4	213.4	484.6		3.6	6.0	4.7	4.76	2	144	144	144	20.4
22.8	256.8	106.2		6.1	7.4	5.4	6.3	2	125	125	125	18.2
23.7	260.7	0.3		4.1	23.9	3.3	10.41	2	74	74	74	11.4
30.1	331.1		44.1	5.6	26.3	9.3	13.71	2	70	68	69	11.0
15.4	169.4		6.4	7.4	14.8	21.4	14.26	2	34	36	35	5.4
37.3	410.3		122.3	6.4	34.9	17.6	19.63	2	60	58	59	9.5
25.2	277.2	84.8		11.3	24.3	8.3	14.63	2	54	54	54	8.6
16.1	177.1	162.9		11.3	17.7	8.8	12.6	2	42	40	41	6.4
17.5	192.5	175.5		10.5	18.5	7.9	12.3	2	45	45	45	7.1
24.9	273.9	177.1		11.4	21.8	11.4	14.6	2	53	53	53	8.5
31.0	341.0	154.0		12.2	21.8	15.0	16.33	2	59	59	59	9.5
27.0	290.0	171		16.0	18.8	15.0	16.6	2	51	51	51	8.13
29.2	321.2	191.8		15.1	24.3	18.0	19.2	2	48	48	48	7.6
32.8	360.8	210.2		16.9	27.6	14.7	19.73	2	52	52	52	8.3
34.2	376.2	215.8		15.3	25.7	15.9	18.96	2	57	55	56	9.0
18.3	200.8	388.2		14.9	28.0	16.7	19.86	2	30	31	30.5	4.6
22.9	251.9	369.1		13.5	25.5	19.3	19.43	2	38	38	38	5.9
19.4	213.4	292.6		10.6	25.3	20.0	18.63	2	35	33	34	5.2
22.3	245.3	368.7		11.8	27.3	21.7	20.43	2	35	36	35.5	5.45
18.8	206.8	433.2		10.9	22.6	18.7	17.4	2	35	35	35	5.4
18.2	200.2	461.8		11.3	16.9	22.6	16.93	2	34	36	35	5.4
18.6	204.6	428.4		12.1	14.7	17.5	14.76	2	40	40	40	6.3

Table VII - B

Thyroxine 4×10^{-6} gm
Cortisone 5 mg. / Kg.

Experimental Day	Animal Weight in grams			Food Intake in grams			Avg. Food N_2 Intake in mg.	Avg Urine N_2 Output in mg	Estimated Hair + Fecal N_2 in mg.	Total N_2 Output in mg.
	#52	# 54	Average	#52	# 54	Average				
Start	339.4	337.3	338.35							
1	349.4	340.2	344.8	27.9	23.8	25.85	968	495	49.5	544.5
2	351.4	341.6	346.5	28.5	24.1	26.3	984	485	48.5	533.5
3	354.6	341.4	348.0	29.1	25.3	26.7	1000	393	39.3	432.3
4 ●	339.3	328.9	334.1	14.8	16.8	12.8	472	432.5	43.3	475.8
5	336.8	334.2	335.5	24.3	18.2	21.25	785	358	35.8	393.8
6	341.9	337.9	339.9	23.8	16.2	20.0	736	372	37.2	409.2
7 ■	330.1	329.9	330.0	0	4.8	2.4	88.4	265	26.5	291.5
8	315.4	321.7	318.5	0	10.1	5.05	186	222	22.2	244.2
9	310.9	315.4	313.15	9.1	8.0	8.55	314	139	13.9	152.9
10	306.6	311.4	309.0	9.4	8.1	8.75	322	138	13.8	151.8
11	304.5	303.9	304.2	10.3	6.5	8.4	309	373	37.3	410.3
12 ▲	296.5	305.4	300.95	11.5	14.7	13.1	483	625	62.5	687.5
13	286.4	304.4	295.4	6.4	15.7	11.05	407	372	37.2	409.2
14 ◆	272.6	300.6	286.6	3.5	16.5	10.0	368	456	45.6	501.6
15	262.3	302.3	282.3	0	16.9	8.45	311	348	34.8	382.8
16	246.4	307.5	276.95	0	20.7	10.35	381	414	41.4	455.4
17	234.9	306.1	270.5	0.3	19.6	9.65	355	404	40.4	444.4
18	221.2	305.9	263.55	0	19.8	9.9	364	343	34.3	377.3
19	215.4	304.9	260.15	7.0	19.6	13.3	490	426	42.6	468.6
20	222.4	303.3	262.85	16.8	21.4	19.1	704	496	49.6	545.6
21	228.7	300.9	264.55	24.1	20.9	22.5	830	536	53.6	589.6
22	230.2	304.4	267.3	24.0	21.9	22.95	846	556	55.6	611.6
23	238.8	301.7	270.25	24.2	22.3	23.25	857	466	46.6	512.6
24	244.4	306.4	275.4	23.6	23.7	23.65	871	570	57.0	627.0

● Day of Thyroidectomy
■ Day of Bilateral Adrenalectomy
▲ Day Medication Begun
◆ Day of Stress

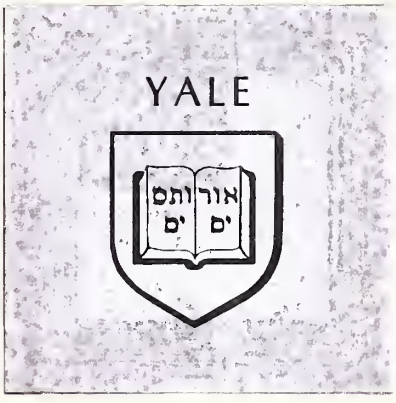
Table VIII - A

Thyroxine 12×10^{-6} gm.
Cortisone 2.5 mg/Kg.

Experimental Day	Nitrogen Balance in mg.		Urine Volume in ml.			Dilution Factor	Klett - Summerson Readings			N_2 in mg/ml of urine as diluted
	Positive	Negative	#52	#54	Average		1	2	Average	
start										
1	423.5		14.8	9.6	13.2	2	129	129	129	18.7
2	450.5		17.8	9.7	13.75	2	119	119	119	17.6
3	567.7		18.0	8.6	13.3	2	96	94	95	14.7
4		3.8	18.7	10.7	14.7	2	95	95	95	14.7
5	391.2		32.3	8.2	20.25	2	55	55	55	8.8
6	326.8		18.7	7.1	12.9	2	93	93	93	14.4
7		203.1	13.3	10.9	12.1	2	68	70	69	11.0
8		58.2	18.1	8.9	13.5	2	51	51	51	8.2
9	161.1		27.8	14.2	21.0	2	23	23	23	3.3
10	176.2		15.9	16.9	16.4	2	28	28	28	4.2
11		101.3	42.4	21.0	31.7	2	38	38	38	5.9
12		204.5	28.7	25.1	31.9	2	60	60	60	9.8
13		2.2	32.8	18.2	25.5	2	45	47	46	7.3
14		133.6	26.2	22.9	24.55	2	58	58	58	9.3
15		71.8	11.1	18.4	14.75	2	74	74	74	11.8
16		74.4	7.4	21.5	14.45	2	92	92	92	14.3
17		89.4	5.4	25.4	15.4	2	85	83	84	13.1
18		13.3	5.2	26.3	15.75	2	68	68	68	10.9
19	214		10.7	22.6	16.65	2	82	82	82	12.9
20	158.4		18.9	26.6	22.75	2	68	68	68	10.9
21	240.4		23.2	24.2	23.7	2	72	70	71	11.3
22	234.4		23.9	24.0	23.95	2	73	73	73	11.6
23	344.4		23.4	18.2	20.8	2	70	70	70	11.2
24	2440		29.9	20.1	25.0	2	71	73	72	11.4

Table VIII - B

Thyroxine 12×10^{-6} gm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